THE
BENZOYLATION OF MALIC ACID

Thesis

by

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THE BENZOYLATION OF MALIC ACID.

Introduction. The work in connection with the benzoylation of malic acid was done mainly to straighten out difficulties in a more comprehensive research problem. It is first necessary, therefore, to outline the other problem, which is to obtain evidence to support or disprove the Huggins theory concerning the structure of Benzene and its derivatives. The Huggins theory (1), a modification of the theory advanced by Vaubel, states that the correct structure of the compound Benzene is as shown (Fig.1) instead of the generally accepted theory advanced by Kekule. If then, the Huggins theory is correct it would be possible to prepare a derivative of Benzene that would be resolved by suitable means into two optically active forms.

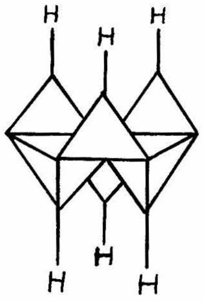

Fig.1

The first problem was to prepare such a compound and resolve it, if possible, into its two optically active forms. The successful carrying out of this procedure would yield evidence in favor of the Huggins theory. Carefully obtained negative results would tend to disprove it.

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(1) M.L.Huggins. Science 55. 679-80. 1922. also J.A.C.S. 44. 1607-17. 1922.

This problem has been attacked from another angle by other investigators. The substances they used were mainly salts of various acids. Le Bel (1) attempted to resolve o-toluidine. Lewkowitsch (2) tried m-methyl salicyclic acid and o-methyl m-hydroxybenzoic acid. V.Meyer and Luhn (3) used nitro- and aldehyd-thymotic acids. None of these attempts were successful.

From various sources (4) evidence was obtained which showed that for the reagent containing the Benzene ring and one that would be unsymetrical according to the Huggins theory, m-nitrobenzoyl chloride would be very good for the following reasons :

1) The ester formed would very likely be a crystalline solid which would be easy to work with.

2) The compound had been prepared by myself in fairly good quantity and was quite pure according to data obtained by other investigators.

3) The reaction between it and a hydroxy group of an alcohol or hydroxy acid would take place readily and quantitatively. It was this point however that had to be cleared up because malic acid was chosen as the other reagent since an optical form of this compound could be obtained pure.

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(1) Le Bel. Bull. Soc. Chem. 38, 98. 1882.

(2) Lewkowitsch. Trans. Chem. Soc. 53, 781. 1888.

(3) V.Meyer and Luhn. Ber. 28, 2795. 1895.

(4) E.E.Reid. J.A.C.S. 39, 124-36.

Other points in favor of the use of malic acid were:

1) It is very soluble in water and therefore an excess could be used to insure complete reaction and easily removed.

2) Although Mandelic acid has a much higher specific rotation than the Malic acid it is possible that the addition of another benzene ring would have a balancing effect on the molecule and hence the rotation of the ester prepared would have a lower rotation than the mandelic acid itself. On the other hand, malic acid has a low specific rotation and the addition of a benzene ring would tend to increase it.

The work as originally outlined was to prepare pure l malic acid, treat it with m-nitrobenzoyl chloride to obtain m-nitrobenzoyl l malic acid, and then attempt by careful fractional crystallization and examination of the solutions with a polariscope, to separate the optical isomers if there were any. Another possible method of separation was to make use of the fact that optically active substances containing nitrogen may be separated by the action of suitable yeasts. If a yeast can be made to grow in a solution of the compound being resolved, it is found that only one isomeric form is used up by the yeast and the solution remaining after separation from the yeast contains an excess of the other form which can easily be detected by means of the polariscope.

Attempts were first made to obtain l malic acid. The method of Darmois and Perrin (1) was used first but the results were so meager that another method was turned to.

The method of H.D.Dakin (2) was used with more success. The final preparation of l malic acid was so slow however that it was deemed advisable to first investigate the reaction between the inactive malic acid and benzoyl chloride. It was found that there was no such compound as benzoyl-malic acid to be found in any of the literature up to 1925. Also, the first attempt to produce this compound was a failure so that it was necessary to concentrate more on this reaction while the l malic acid was being produced. Since most of the research deals with the benzoylation of malic acid to produce benzoyl-malic acid, the work done in obtaining the l malic acid will be described very briefly.

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(1) Darmois and Perrin. Bull. Soc. Chem. 35, 353-7. 1924.

(2) H.D.Dakin. J.Biol.Chem. 59, 7. 1924.

EXPERIMENTAL WORK

<u>L Malic acid.</u> The resolution of malic acid by the method of Darmois and Perrin was not successful since there was no separation of ammonium molybdo-malate into antipodes.

The method of H.D.Dakin was presumably successful but the final product was not examined for purity because the available time was used in investigating the benzoylation of i malic acid.

<u>Benzoylating i Malic acid.</u> The only similar compound that has been prepared is the acetyl-malic acid which was prepared by heating acetyl chloride with malic acid to form acetyl-malic acid anhydride (1), which becomes acetyl malic acid when treated with water.

The work with Benzoyl chloride and malic acid was begun in much the same way. Various methods were tried in order to get the best conditions for the reaction and subsequent handling of the product. The reaction was limited as far as possible to the following :

$$C_6H_5COCl + COOH.CH_2.CHOH.COOH = C_6H_5COOC_4H_5O_4 + HCl$$

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(1) Beilstein I, 743. (3rd.Ed.)

1) A mixture of 14 g. (0.1 mol.) Benzoyl chloride and 15 g. i malic acid (13.4 g. equals 0.1 mol.) in 100 cc. of benzene, was refluxed for two hours without any evolution of HCl showing that the reaction does not take place under these conditions. In this test it was desired to keep the temperature low and also provide the mixture with agitation.

2) A mixture of 7 g. (0.05 mol.) Benzoyl chloride and 6.7 g. (0.05 mol.) of i malic acid were heated by means of a water bath in a flask fitted with a small reflux condenser. After the temperature was at 100° for a few minutes the mixture began to react as evidenced by the evolution of HCl. The reaction took place quite nicely and after about two hours was complete. Some malic acid was left but the rest was in what had been the liquid benzoyl chloride layer. The water bath was then removed and the substances were heated with a very small flame. Some substance bublimed into the neck of the flask in fine leaflets (Sample 1). The residue was dissolved in a little hot water from which it crystallized at about 70°. More water was added and the solution was brought to boiling. Upon cooling, crystals came out at about 50°. (Samples 2 and 3). The crystals were fine leaflets. The solution was put in a desicator and after several weeks another type of fine crystals was obtained. The quantity obtained was too small to work with.

	1.	2.	3.
Melting point -----	118°-120°	119°-120.3°	119°-120° unc.

The melting point confirms the fact that the samples were Benzoic acid.

3) A mixture of i Malic acid and Benzoyl chloride in molal proportions was heated on a water bath as before. When the reaction seemed complete except for a slight amount of Malic acid another molal proportion of Benzoyl chloride was added and the mixture was again heated on the water bath. The rest of the Malic acid then reacted. The mixture upon cooling became a crystalline mass containing Benzoyl chloride. This was removed by washing the mass well with Benzene. The residue was washed several times with hot water and a sample taken (1). The mass was dissolved in 50 cc. of hot water and brot to boiling when it it dissolved completely. The solution was allowed to stand overnight in a dessicator and crystallized out in large clusters of needles which were removed and washed with water. A sample (2) was taken for melting point determination.

	1.	2.
Melting point. --------	126°-128° unc.	109°-110° unc.
		107°-108° unc.

Owing to the fact that if Sample 2. were heated slowly it would melt at about 109° whereas if it were melted rapidly (a tube containing the substance was put in the hot bath) it would melt at about 90° it was decided that the substance was either hygroscopic or it contained water of crystallization.

Some of the crystals were dissolved in Acetone and were found to be too soluble to crystallize well.

4) The Baumann-Schotten reaction (1) was also tried with i Malic acid and Benzoyl chloride in the presence of Sodium hydroxide. The proportions of each were : 1 mol of malic acid, 2 mols of Benzoyl chloride and 6 mols of NaOH. This indicates that there was no reaction between the Malic acid and the Benzoyl chloride; the NaOH reacting as follows:

$$\mathrm{COOH.\ CH_2.CHOH.COOH} \quad \mathrm{2\ NaOH} = \mathrm{COONa.CH_2.CHOH.COONa} \quad \mathrm{2\ H_2O}$$

$$\mathrm{2\ C_6H_5COCl} \quad \mathrm{4\ NaOH} = \mathrm{2\ C_6H_5COONa} \quad \mathrm{2\ NaCl} \quad \mathrm{2\ H_2O}$$

whereas, if benzoylation had taken place, a smaller number of mols of sodium hydroxide would be required to neutralize the acids.

5) A mixture of i Malic acid and Benzoyl chloride in molal proportions was treated as in (2). When the reaction was complete, as shown by the evolution of HCl, the product was poured into a beaker, dissolved in a minimum of hot water and boiled to use up any unreacted Benzoyl chloride. Upon cooling to about 50°, Benzoic acid precipitated out and was removed. The filtrate was allowed to stand for several days. A few crystals of a mixed nature were obtained, (1) and (2), and removed. Upon further standing, the solution yielded crystalls, which, after two recrystallizations, became large needles like Sample 2, (3). For convenience this substance shall be called "X".

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(1) Baumann. Ber. 19, 3219.

The melting points of these samples were as follows :

	1.	2.	3.("X")
Melting point. ----	119.5°- 120.5°	115°- 120°	109°-111°
			110°-110.7° unc.

The solubility of "X" was tested with Benzene. The substance apparently **did not** dissolve in cold Benzene and was only sparingly soluble in boiling benzene.

6) A mixture of 26.8 g. (0.2 mol.) of i Malic acid and 42. g. (0.3 mol.) of Benzoyl chloride were treated as in (3). All but about one gram of the Malic acid reacted. The mixture was then cooled, broken up and treated with Benzene to remove the excess Benzoyl chloride or any Benzoic acid. The solid was then dried. Yield -- 30 g. (This batch was lost.)

7) The above proportions were used and treated as before except that 0.2 mols of excess Benzoyl chloride were added toward the end of the reaction instead of the 0.1 mol. This makes the product a little easier to handle. The yield of crude "X" after treatment with Benzene was 29 g. (Approximately the same as before.) After two recrystallizations a very good quality of crystals was obtained. With these crystals and those obtained from (5) the analytical work was done.

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ANALYTICAL. From consideration of the physical properties of known substances which might possibly result from the decomposition of Malic acid by Benzoyl chloride which is a very strong dehydrating agent, it can readily be seen that the compound "X" is a different substance.

	M.P.	Sol. in H_2O
Malic acid. ($COOH.CH_2.CHOH.COOH$)	130°	Very soluble.
Benzoic acid (C_6H_5COOH)	120°-121°	Very slightly soluble
Fumaric acid ($HOOC.CH:CH.COOH$)	286°-287°	Very slightly soluble
Maleic acid ($HOOC.CH:CH.COOH$)	130°	Very soluble
Maleic anhydride ($C_4H_2O_3$)	56°-57°	....
Compound "X"	110°-116.7°	Soluble.

Since the substance "X" could not be heated in an oven without changing the nature of it, it was found necessary to make the analyses on samples well dried in air.

I. The equivalent weight of "X" was found by titration. A group of air-dried samples was titrated against NaOH, using Phenolphthalein as indicator.

	Wt. Sample.	cc. N/10 NaOH.	Equiv. Wt. "X"
1)	0.1355 g.	11.44	118.5
2)	0.4836 g.	40.40	119.5
3)	0.4472 g.	37.00	120.5

Average equivalent weight ---------------------- 119.5

II. Some of the best crystals (same as I) were air dried to find the equivalent weight by titration.

	Wt. Sample.	cc. $^N/10$ NaOH.	Equiv. Wt "X".
1)	0.2880 g.	23.7	121.5
2)	1.7832 g.	146.98	121.3

Average equivalent weight ------------ 121.4

III. Two samples were heated in an oven at 90° for 48 hours or to constant weight in order to determine the water of hydration.

	1.	2.
Weight of sample --------- (Before heating)	2.3477 g.	3.2331 g.
Weight after heating ------	2.2590 g.	3.1109 g.
Weight of water lost ------	0.0887 g.	0.1222 g.
Per cent of H_2O in sample --	3.78%	3.78 %

If one molecule of "X" were losing one molecule of water the molecular weight of this method would be equal to the quantity :

$$\frac{\text{Molecular weight of the water}}{\text{Per cent of water found.}}$$

Apparent molecular weight of "X" ------- 476.

A melting point of this dehydrated substance was taken and found to be quite sharp.

Melting point --------------------- 137.5°- 138.5°

IV. The equivalent weight of the above material after dehydration was found by titration.

	Wt. Sample.	cc. N/10 NaOH	Equiv. Wt. "X"
1)	0.2476 g.	21.25	116.6
2)	0.3605 g.	31.00	116.2
3)	0.5109 g.	43.70	116.7

Average equivalent weight ---------------- 116.5

V. After titration the solutions from (II) were combined and an analysis was made to find the Benzoic acid content of "X". The solution was saponified by boiling with 150 cc. of the standard Sodium hydroxide solution in a reflux apparatus for 6 hours. The solution was then titrated with 0.1106 n. HCl solution.

cc. NaOH necessary to titrate original solution --------------------	170.7 cc.
cc. NaOH used for saponification ----------------	150.0 cc.
cc. HCl used for excess NaOH ---------------------	57.5 cc.
Equivalent cc. NaOH : 57.5 x 1.106 --------------	63.60 cc
cc. NaOH used up in saponification: 150 − 63.6 =	86.4 cc
Theoretical volume of NaOH used for saponification $= \frac{170.7}{2} =$	85.3 cc

It can be seen that the last two values check to within one per cent so that the fact is well established that the compound contains one Benzoyl radical for every two carboxyl groups.

The solution was then evaporated to about 100 cc., cooled and treated with 50 cc. conc. HCl (excess). The Benzoic acid that was precipitated was then extracted repeatedly with chloroform until it was all removed. (Some of the extract was lost so that the resulting Benzoic acid found was low.) The chloroform was then allowed to evaporate, after which the dish containing the residue was allowed to stand in a dessicator for four hours.

The weight of Benzoic acid found was ------------ 0.941 g.

Molecular wt. of Benzoic acid is ----------------- 122

Weight of the sample used ----------------------- 2.071 g.

$$\frac{\text{Molec. Wt. of BzOH}}{\text{Molec. Wt. of "X"}} = \frac{0.941}{2.071}$$

Molec. Wt. of "X" --- 268

Per cent Benzoyl from the formula ------- 43.1 %
$(C_6H_5CO\text{-}O.CH.COOH.\ CH_2.COOH)$

Per cent Benzoyl by Benzoic acid obtained --- 39.1 %

There are several reasons why this result is high, (Molecular weight of "X"). If there were any moisture left on the crystals after the air drying the resulting proportion of Benzoic acid would be low. Also some of the chloroform extract was lost which would further lower the amount of acid found. These two effects are cumulative and from the expression for finding the molecular weight it can be seen that the lower the value found for Benzoic acid the higher would be the calculated molecular weight of the compound.

VI. An approximate molecular weight determination was made by determining the freezing point lowering with an aqueous solution. The solution was made up at about 0.2 N in "X" and added to ice in a Dewar bulb. The melting point of ice was observed before addition and when the freezing point became constant after addition of "X", a 10 cc. sample was carefully withdrawn, and titrated. Since a concentration of 1.0 molal will lower the freezing point of water 1.86 degrees the molality can readily be calculated. The normality is found by titration so that the number of carboxyl groups and the approximate molecular weight can be calculated.

	1.	2.	3.
Freezing point lowering	.158°	.192°	.180°
0.1 N NaOH, titration	14.85cc.	19.3cc.	17.5cc.
Norm. "X", titration	.1485	.193	.175
Molality by Fr. pt. low.	.0850	.103	.0968
Molec. Wt. "X"	211	226	219

Average molecular weight of "X" -------- 219

(Note. This assumes the value of 121 previously found by titration to be correct.)

This experiment proves conclusively that the molecular weight is twice the equivalent weight, and that the substance "X" has two carboxyl groups.

From the above results an approximate ionization constant was calculated. Neglecting the second ionization the ionization constant appears to be of the order of 1×10^{-3}.

RESULTS. The results of the experiments are as follows:

I. By titration, the compound "X" was found to have an equivalent weight of 119.5 and 121.4 Average ------------- 120.5

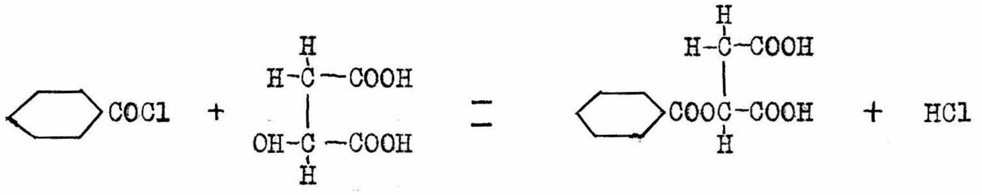
II. From loss of water the apparent molecular weight of "X" was found to be 476.

III. It was proved by saponification and titration that there was one Benzoyl radical for every two carboxyl groups.

IV. It was proved by freezing point lowering experiments that there were two carboxyl groups to the molecule.

CONCLUSIONS

If the reaction between the Malic acid and Benzoyl chloride takes place in the usual manner the following should be true :

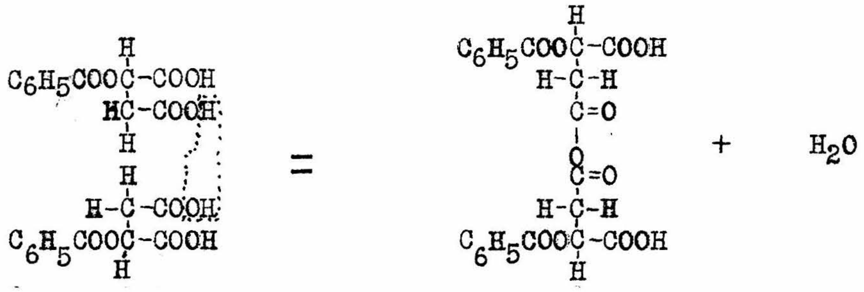


This corresponds to the formula $C_{11}H_{10}O_6$, Molec Wt. --- 238.

The results of the analytical work therefore show quite conclusively that this compound has been prepared.

The compound obtained was shown to have a molecular weight of approximately 240, that there was one Benzoyl radical to every two carboxyl groups, and that the compound contained only two carboxyl groups. This corresponds to the structure of the compound Benzoyl-malic acid which shows that the reaction takes place as indicated.

Another conclusion can be drawn very readily from the results obtained in the loss of water determination. If two molecules of Benzoyl-malic acid lose one molecule of water to form an anhydride thus :



the experiment gives an exact determination of the molecular weight. From the above reaction it can be seen that the indicated molecular weight would be twice the molecular weight of the Benzoyl-malic acid itself, or 476. This is exactly the result that was obtained in this experiment. by loss of water.

The results of the titrations on the crystalline and dehydrated samples also show the loss of one molecule of water.

$4 \times 121 = 484$

$4 \times 116.5 = 466$

The difference equals 18. or one H_2O.

Since the anhydrous form shown would be easily hydrolysed when dissolved in water and titrated with a base it is reasonable to suppose that a compound of this type was formed when the samples were heated.

This being the case the molecular weight of the compound formed is proved conclusively.

The compound is best prepared in the following manner : Heat a mixture of Malic acid and Benzoyl chloride in molal proportions in a flask fitted with a short reflux condenser, by means of a water bath. At 100° the reaction takes place very readily and continues until the liquid clears and no more HCl is evolved. If any Malic acid remains, add another molal portion of Benzoyl chloride. When the evolution of HCl ceases the solution is cooled and the solid broken up, removed, crushed and washed repeatedly with Benzene. The solid is then dried, washed well with several small portions of hot water and crystallized. Yield ------ about 65 %, calculated on Malic acid.

Work on the benzoylation of Malic acid was done under the direction of Prof. H. J. Lucas whose assistance I gratefully wish to acknowledge here. I also wish to acknowledge the help of E.W.Hart on freezing point determination; of Warren P. Baxter in connection with calculations of molecular weight and ionization constant from the freezing point lowering; and of L.C.Pauling in determination of the crystal structure.

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PHYSICAL PROPERTIES OF BENZOYL-MALIC ACID.

Benzoyl-malic acid is a colorless, crystalline solid. When pure it crystallizes from water in clusters of long, hard needles which are monoclinic, pseudohexagonal in structure.

Melting point --------------- $110^{\circ} - 110.7^{\circ}$ (unc.)

Solvent		Solubility.
Water (0°)	----------------	3.5 g. in 100 cc.
Water (100°)	---------------	very soluble.
Benzene	-----------------	Very slightly soluble
Acetone	-----------------	very soluble.
Acetic acid	-----------------	very soluble.

Upon prolonged boiling of a solution of Benzoyl-malic acid the substance is hydrolized to Benzoic acid and Malic acid.

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SUMMARY

I. The resolution of i-Malic acid by the method of Darmois and Perrin was not successful.

II. Benzoyl Chloride reacts when heated with i-Malic acid at 100°, with evolution of hydrogen chloride.

III. In Benzene solution, Benzoyl chloride does not react with i-Malic acid, even at the boiling temperature.

IV. In the presence of aqueous sodium hydroxide, Benzoyl chloride and i-Malic acid do not react.

V. The product of the reaction between Benzoyl chloride and i-Malic acid is a solid which crystallizes from water in clusters of long, hard needles, which are monoclinic in structure.

VI. This compound is i-Benzoyl-malic acid, whose formula is : $C_6H_5COOCH.COOH.CH_2.COOH$. Molec. Wt. ----- 238 .

VII. When dried at 90° two molecules of i-Benzoyl-malic acid appear to lose one molecule of water to form an anhydride.

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